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BIO-FABRICATION OF SILVER NANOPARTICLES FROM *VIGNA MUNGO* SEED EXTRACT AND ITS EFFECT ON THE FUNGUS, *FUSARIUM OXYSPORUM IN VITRO*

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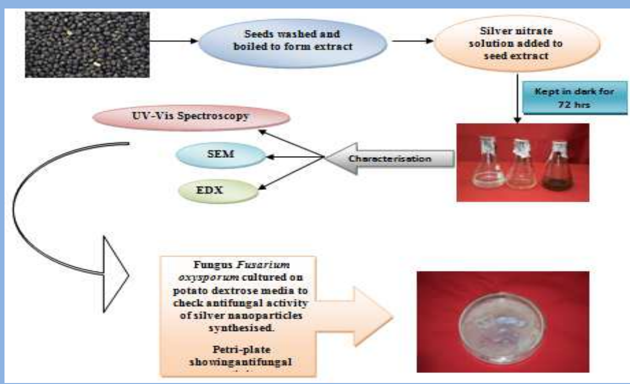
ABSTRACT

Silver nanoparticles are being widely used and obtaining great attention in the field of microbiology and agriculture. The present experiment was performed to bio-fabricate silver nanoparticles from Vigna mungo seed extract and to appraise it's the antifungal property. Results showed that the silver nanoparticles have a great absorption at 440nm and their characterization of size and shape was done by SEM analysis. The biofabricated 20nm, spherical nanoparticles exhibited antifungal properties and inhibited the growth of fungus, Fusarium oxysporumin vitro.

GRAPHICAL ABSTRACT



KEYWORDS*: *Biofabrication, Fusarium Oxysporum, Green Synthesis, Silver Nanoparticles & VIGNA Mungo

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Original Article

INTRODUCTION

In the present scenario, nanoparticles are gaining attention and interest because of their broad range of application. Nanoparticles are used in various areas such as in cosmetics industries, biolabeling, catalysis, chemistry, medical sciences and in microbiology, exhibiting antimicrobial properties against fungi and bacteria (Sivanesan et al., 201l; Zhu et al., 2013; Liu et al., 2013; Ismail et al., 2013; Zhang et al., 2013; Yehia and Al-Sheikh, 2014; Medda et al., 2015). Metal nanoparticles exhibit antimicrobial activities and show large surface to volume ratio because they disrupt the cell wall morphology of the microorganism by interacting with the cell membrane (Trop et al., 2006; Ahmed et al., 2013). Among, the metal nanoparticles utilized for various purposes silver is widely used and most commercialized nanoparticle due to its productivity and non-toxicity to humans (Rai et al., 2009). Larue et al., 2014 reported production of 5,000 tons of silver nanoparticles per year and these figures are assumed to enhance in the coming years. There are several methods for the formation of nanoparticles,

which includes both chemical synthesis and microbe-mediated synthesis. Chemical Synthesis is fast, but expensive and is toxic to both humans and the environment. Microbe synthesis is not feasible because of the high cost of maintenance of asepticlaboratory condition. These drawbacks of the above-mentioned methods forced the scientists and researchers to discover plants which have potential to form nanoparticles and to develop an eco-friendly, green and sustainable approach (Savithramma et al., 2011). Green synthesis of nanoparticles is an approach to synthesize nanoparticles using microorganisms such as algae, fungi, bacteria and from different plants and their parts having biomedical applications. This method is eco-friendly, cost-effective, non-toxic to living tissues, benign and a green technology (free of toxic chemicals). Therefore, the utilization of plant extracts (from seed, leaves etc) is gaining significance because of its feasibility, non-toxicity, rapid synthesis and economic practice. Many researchers have synthesized nanoparticles from various plants such as *Boswellia ovalifoliolata* (Ankanna et al., 2011); *Ulva lactuca*(Kumar et al., 2011); Banana (Ibrahim, 2015); *Erythrina indica* (Rathi et al., 2015); *Rosa indica*(Ramar et al., 2015); *Thymbra spicata*(Veisi et al., 2018) and many more. The present study was carried out for the quick and green synthesis of silver nanoparticle from *Vigna mungo* seed extract and to check the function of phytochemical constituents present in seed extract. *Vigna mungo* contains various amino acids, flavonoids, phenolic compounds. Thus, this plant was selected for biofabrication of silver nanoparticles, to assess its antifungal activity against fungus *Fusarium oxysporum* and its effect on seed germination and dormancy.

MATERIALS AND METHODS

Preparation of *Vigna mungo* Seed Extract

Seeds of *Vigna mungo* were first surface sterilized by 0.01% mercuric chloride. 10g seeds were grinded in a pestle and mortar, then mixed in 100ml of distilled water. This solution was heated not boiled for 10 mins. The solution was filtered through Whatman No.1 filter paper and collected in a beaker. The solution was stored in a refrigerator for further use.

Preparation of Silver Nitrate Solution

The silver solution was prepared by adding 1.575g of Silver Nitrate in 1L of distilled water.

Concoction of Silver Nanoparticles Solution

10ml seed extract solution was added in a conical flask containing 90ml of silver nitrate solution. The solution was kept in the dark room for 72 hrs at room temperature for further observation.

Colour change of the solution indicated the synthesis of Ag nanoparticles.

Preparation of Medium for *Fusarium Oxysporum* Culture

Potato Dextrose agar Medium was prepared for the culture of fungus, *Fusarium oxysporum.* 200g potato infusion, 20g dextrose, 20g agar and 1000ml distilled water was taken to prepare the medium and poured in Petriplates for inoculation. The filter paper disc of the size 1x1cm was cut and placed in the poured medium. The fungus was inoculated with needle and 3 drops of the synthesized silver nanoparticles solution was added to the disc. The Petri-plates were placed in the BOD incubator for 1 week at 28^{o}C.

Characterization of Synthesized Silver Nanoparticles

Colour change of solution marked the synthesis of nanoparticles. UV-Vis spectroscopy at 400-700 nm was

recorded because nanoparticles show an absorbance peak in this range SEM and EDX analysis was done of the synthesized Ag nanoparticles. Before analysis the solution was centrifuged at 20,000 rpm, the pellet was collected and dried to form powder.

RESULTS AND DISCUSSIONS

UV-Vis Spectroscopy Analysis

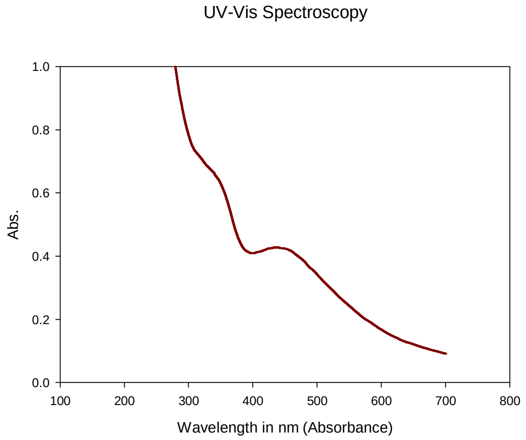

Figure 1: Shows the Absorbance of Synthesized Silver Nanoparticles from *Vigna Mungo* Seed Extract Showing Absorbance at 440nm after 72hrs

After 72 hrs the colour of the seed extract solution changes to reddish brown marking the synthesis of silver nanoparticle as shown in figure 2. The graph was made in Sigma Plot 11.0. The maximum absorbance of Silver nanoparticles was observed at 440nm showing the increase in the synthesis of the particles as in figure 1 (Jayandran et al., 2015; Nayagam et al., 2017). The colour change and observanceare due to the reduction of Silver ion to metallic silver (Karuppiah and Rajmohan, 2013; Ali et al., 2016). Excitation of Surface Plasmon resonance may also correspond to colour change in nanoparticles synthesis as reported by researchers (Narayanan and Sakthivel, 2008; Xiaoming et al., 2009; Medda et al., 2015). Elevated levels of carbohydrates, flavonoids, and steroids in the plant extract cause reduction of Silver and the phytochemicals dispense firmness to nanoparticles (Suna et al., 2014; Ahmed et al., 2016). The rate and amount of production of nanoparticles is influenced by the nature of plant extract, its concentration, pH, temperature and concentration of metal ion (Dwivedi and Gopal 2010; Mittal et al., 2013).

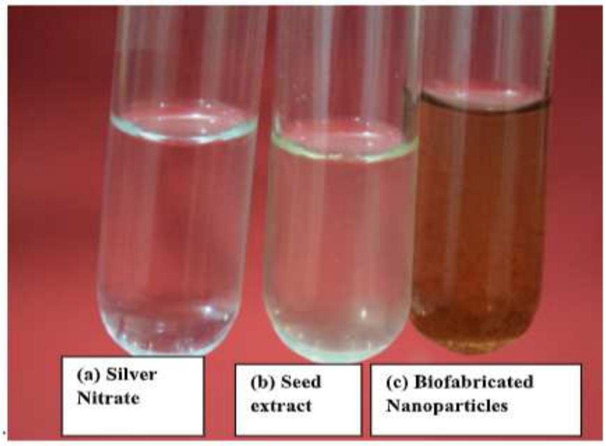

Figure 2: Shows the Colour Change Pattern of Biofabricated Silver Nanoparticles

SEM and EDX Analysis

The integration of techniques such as SEM (Scanning Electron Microscopy) and EDX (Energy Dispersive X-ray Spectroscopy) has played a significant role in characterization of size, morphology and chemical constituent present in nanoparticles. SEM and EDX analysis was done at the University Sophisticated Instrumentation Facility (USIF), Aligarh Muslim University, Aligarh, India. SEM images confirmed the synthesis of nanoparticles of size 20nm and spherical shape (Figure 3). The shape of the silver nanoparticles synthesized is mostly spherical (Janani et al., 2014; Nayagam et al., 2017). EDX analysis showed the atomic % and weight % of the elements present in the nanoparticles and silver have 2.34 and 13.55, atomic % and weight % respectively.

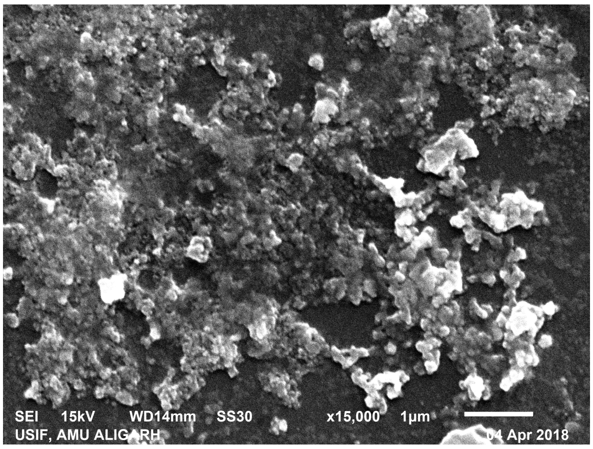

Figure 3: SEM Image of Synthesized Silver Nanoparticles

Table 1: EDX Analysis Showing Atomic % and the Weight % of Elements

Element	Atomic%	Weight%
C	26.27	40.73
N	16.97	22.57
O	20.57	23.95
Na	2.14	1.73
Al	0.67	0.46
Si	9.91	6.57
Cl	0.72	0.38
K	1.03	0.49
Ag	13.55	2.34
Au	8.16	0.77
Au	8.16	0.77
Totals	**100.00**	**100.00**

Antifungal Activity

The bio-synthesized silver nanoparticles prepared from *Vigna mungo* seed extract exhibited antifungal property against fungus, *Fusarium oxysporum.* Our results revealed that the Petri-plate having filter paper disc (dipped in nanoparticles) had no fungal growth on it, but in the control Petri-plate the fungus grew on the filter paper disc (Figure 4). This showed an inhibitory activity of nanoparticle against fungus. Similar results were observed by various other researchers on *Rhizopus* (Narayan and Park, 2014), *Alternaria alternate* and *Botrytis cinerea*(Ouda, 2014), *Aspergillus* (Medda et al., 2015). According to Bragg and Rannie(1974), Thurman and Garba (1989), Kim et al., (2009) silver is highly reactive metal and possess antimicrobial properties. It causes physical damage to microbe and hampers its respiration,

metabolic activities and as well as ion transport (Morones et al., 2005; Pal et al., 2007; Ali et al., 2016). Anomalous shipping of ions results in aggregation of silver ions impeding cellular processes like respiration and metabolism by responding to different molecules. Silver also generates reactive oxygen species (ROS) which are disastrousto cells and damage proteins, nucleic acids and lipids (Storz and Imlay, 1999; Carlson et al., 2008; Hwang et al., 2008; Kim, 2009). According to Ahmed et al., 2016 size, environmental conditions like pH, ionic strength and capping agents are major factors leading to the antimicrobial behaviour of nanoparticles. According to Lamsal et al., 2011antifungal activity of biofabricated nanoparticles not only hampers growth of fungal hyphae, but it also restrains the conidia germination and propagation.

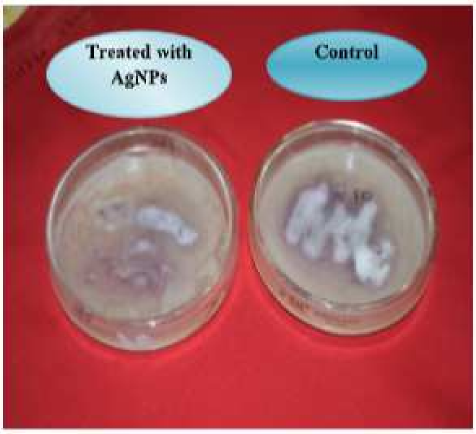

Figure 4: First Petri-Plate Shows Antifungal Activity of Fabricated Silver Nanoparticles and the Second is Control

CONCLUSIONS

The present study focused on the bio-fabrication of Silver nanoparticles from *Vigna mungo* seed extract as a novel method and showed the antifungal activity of silver nanoparticles. This method of bio-fabrication of nanoparticles from the plant extract is safe, inexpensive, energy efficient green technology. This can foster the production and application of silver nanoparticles in the management of various pest diseases in the coming years. It will open a new area and strategies for the eradication of plant disease pathogens without harming the microbiota.

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